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(54) **NON-INVASIVE MEASUREMENT OF BIOLOGICAL ANALYTES**

(71) Applicant: **BioSpex Inc.**, San Jose, CA (US)

(72) Inventors: **Wei Yang**, Los Altos, CA (US); **Leyun Zhu**, Andover, MA (US)

(73) Assignee: **BioSpex, Inc.**, San Jose, CA (US)

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G01N 21/65 (2006.01)
G01N 21/64 (2006.01)
A61B 5/145 (2006.01)
G01N 21/84 (2006.01)

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CPC ***A61B 5/0075*** (2013.01); ***A61B 5/0071*** (2013.01); ***A61B 5/14532*** (2013.01); ***A61B 5/14546*** (2013.01); ***G01N 21/6486*** (2013.01); ***G01N 21/65*** (2013.01); *G01N 2021/6419* (2013.01); *G01N 2021/8466* (2013.01); *G01N 2201/06113* (2013.01)

(58) **Field of Classification Search**

CPC A61B 5/0075; A61B 5/14546; A61B 5/14532; A61B 5/0071; G01N 21/65; G01N 21/6486; G01N 2021/6419; G01N 2021/8466; G01N 2201/06113

See application file for complete search history.

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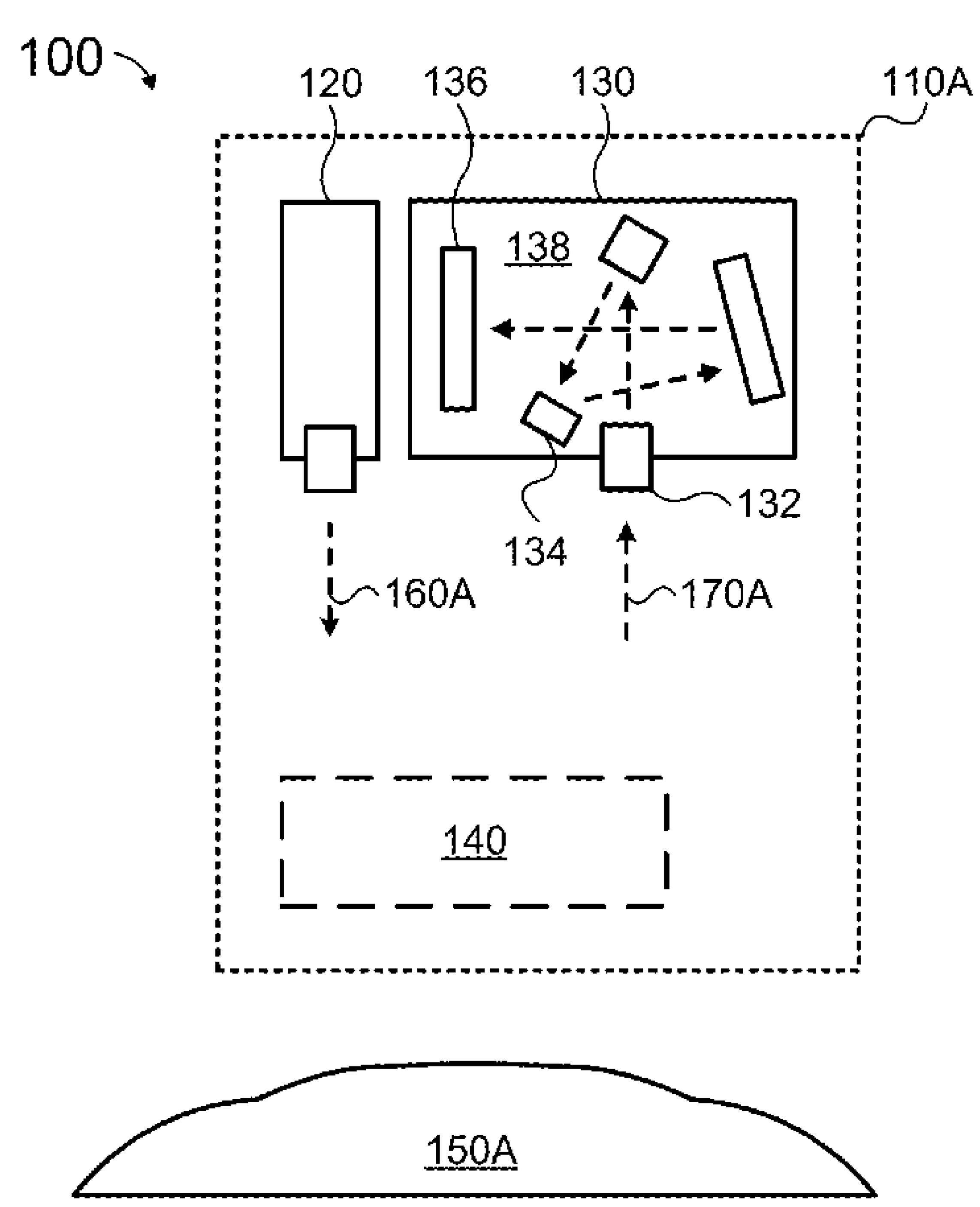
Primary Examiner — Eric F Winakur
Assistant Examiner — Abid A Mustansir
(74) *Attorney, Agent, or Firm* — Carr & Ferrell LLP

(57) **ABSTRACT**

Systems and methods for adjusting for non-invasive measurement of biological analytes. Exemplary methods include: illuminating an analyte using first light, the first light having a first excitation wavelength; detecting a first spectrum from the analyte illuminated by the first light, the first spectrum including a first Raman signal and fluorescence; illuminating the analyte using second light, the second light having a second excitation wavelength; detecting a second spectrum; illuminating the analyte using third light, the third light having a third excitation wavelength; detecting a third spectrum; recovering the first Raman signal using the first spectrum, the second spectrum, and the third spectrum using an inverse transform; and using the first Raman signal to identify and measure at least one molecule of the analyte using a database of identified Raman signals.

20 Claims, 11 Drawing Sheets



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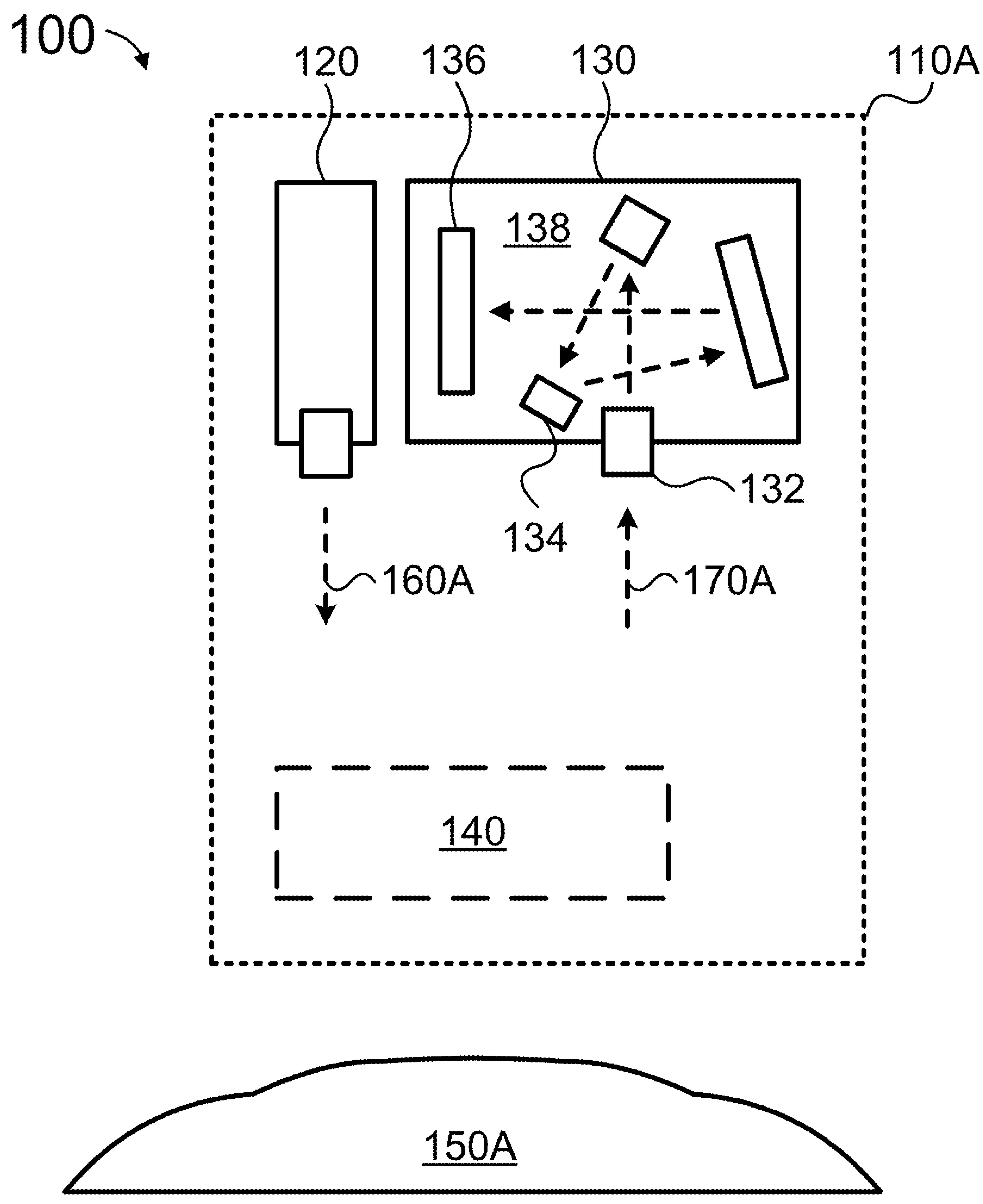


FIG. 1

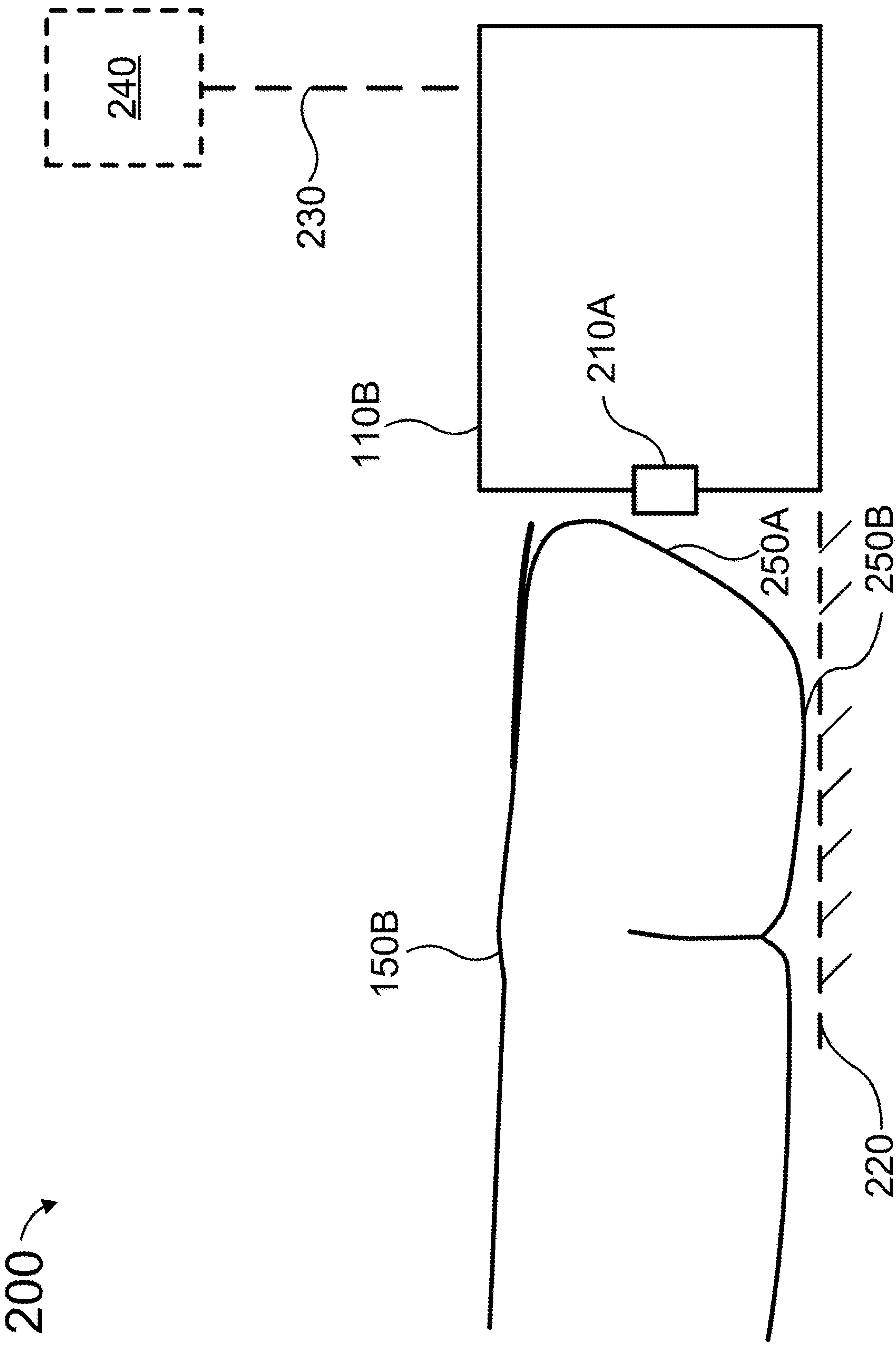


FIG. 2

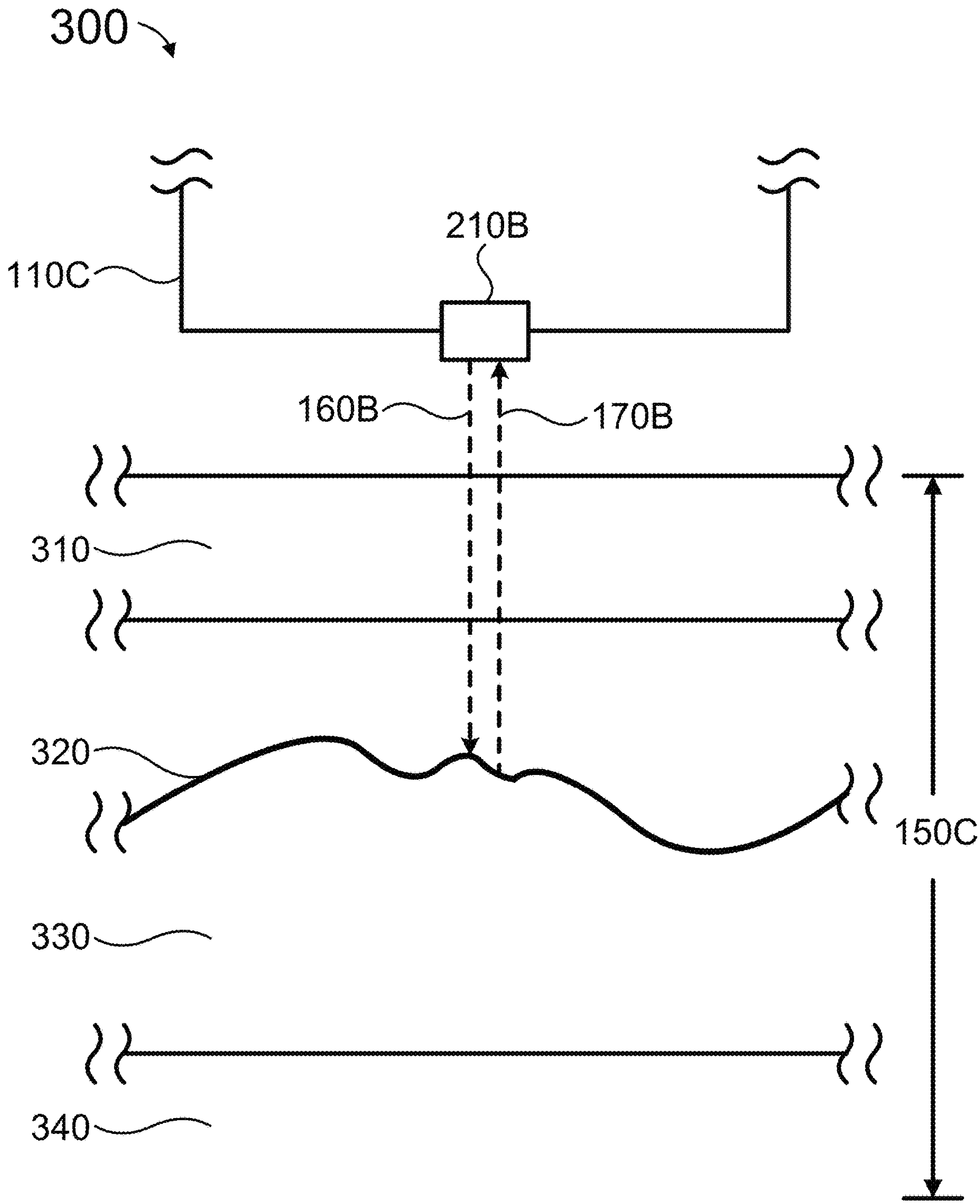


FIG. 3

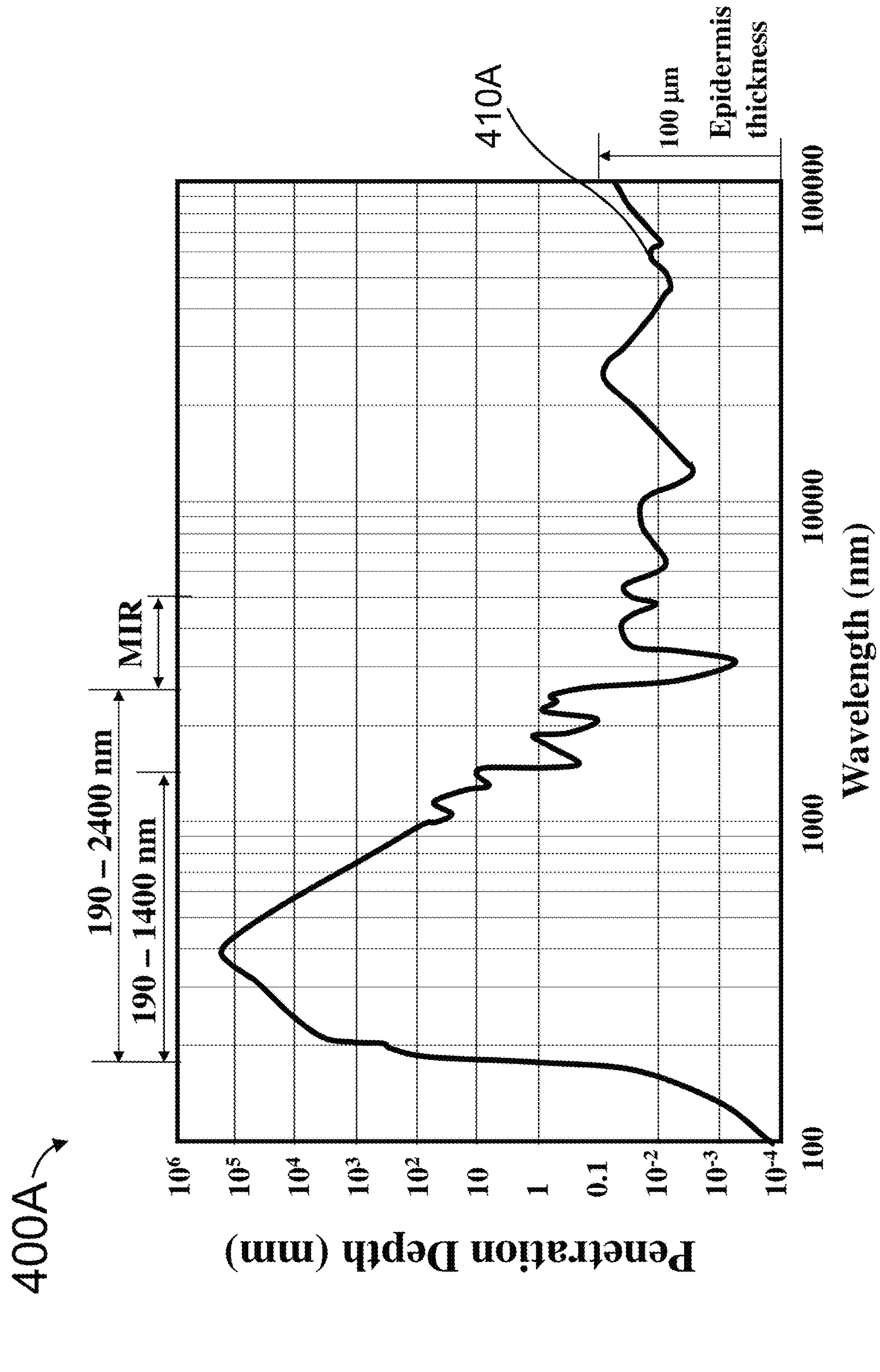

400A
410A
190 – 2400 nm
190 – 1400 nm
MIR
100 μm
Epidermis thickness
Penetration Depth (mm)
10^6
10^5
10^4
10^3
10^2
10
1
0.1
10^{-2}
10^{-3}
10^{-4}
100
1000
10000
100000
Wavelength (nm)

FIG. 4A

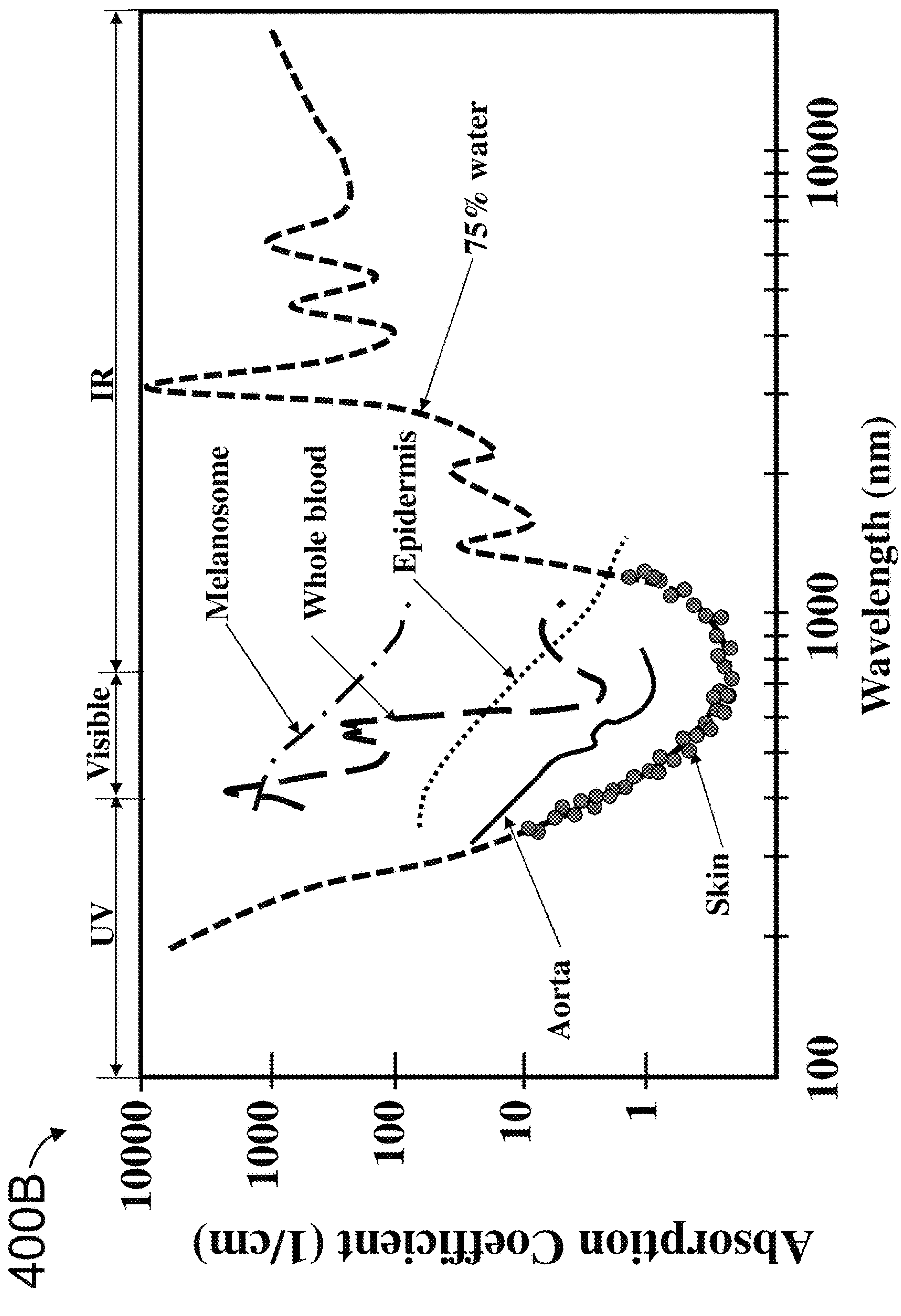
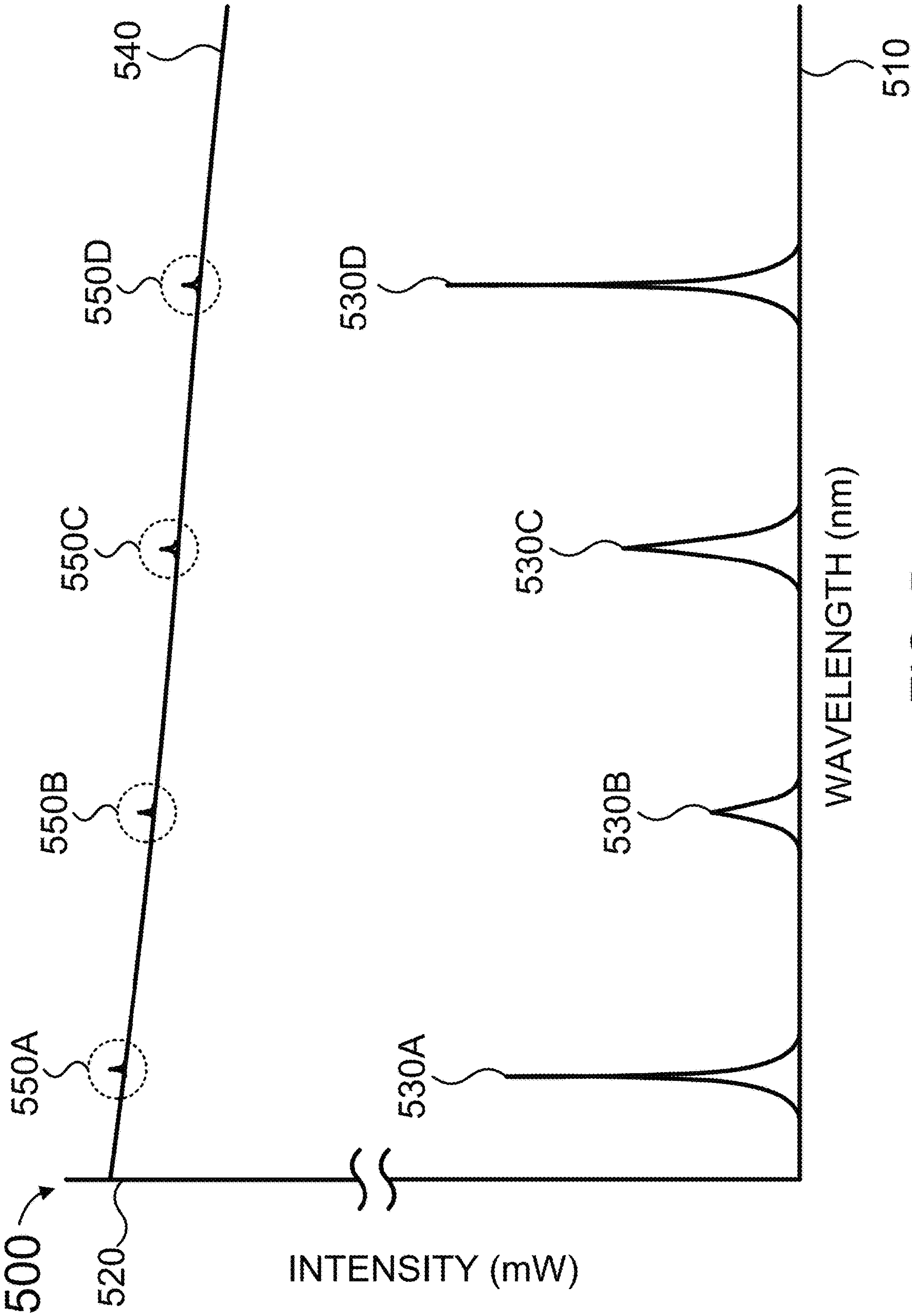

400B
Absorption Coefficient (1/cm)
10000
1000
100
10
1
100
1000
10000
Wavelength (nm)
UV
Visible
IR
Melanosome
Whole blood
Epidermis
75% water
Aorta
Skin

FIG. 4B



500
520
INTENSITY (mW)
WAVELENGTH (nm)
510
540
550A
550B
550C
550D
530A
530B
530C
530D

FIG. 5

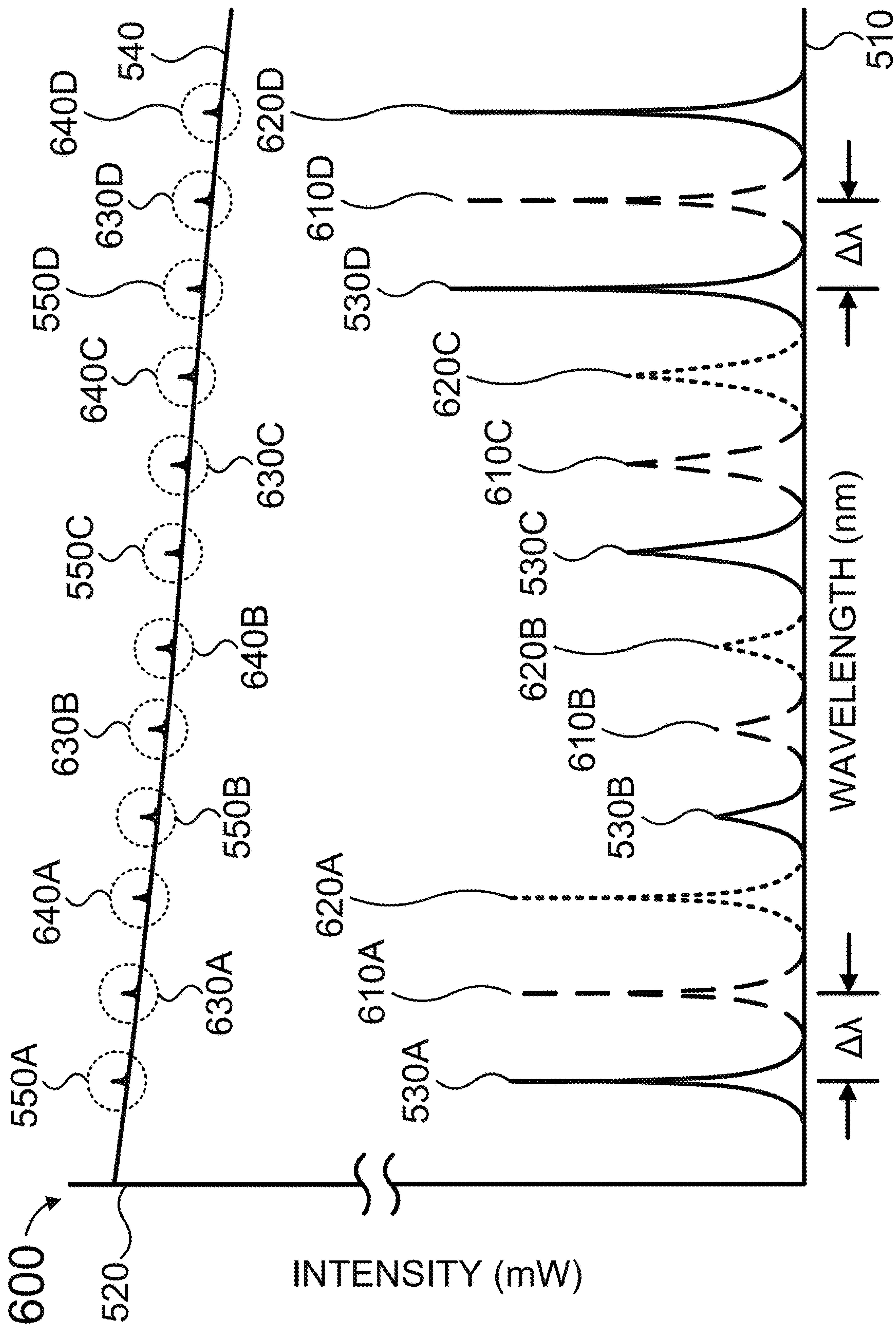

600
520
INTENSITY (mW)
540
510
WAVELENGTH (nm)
550A
630A
640A
550B
630B
640B
550C
630C
640C
550D
630D
640D
530A
610A
620A
530B
610B
620B
530C
610C
620C
530D
610D
620D
Δλ
Δλ

FIG. 6

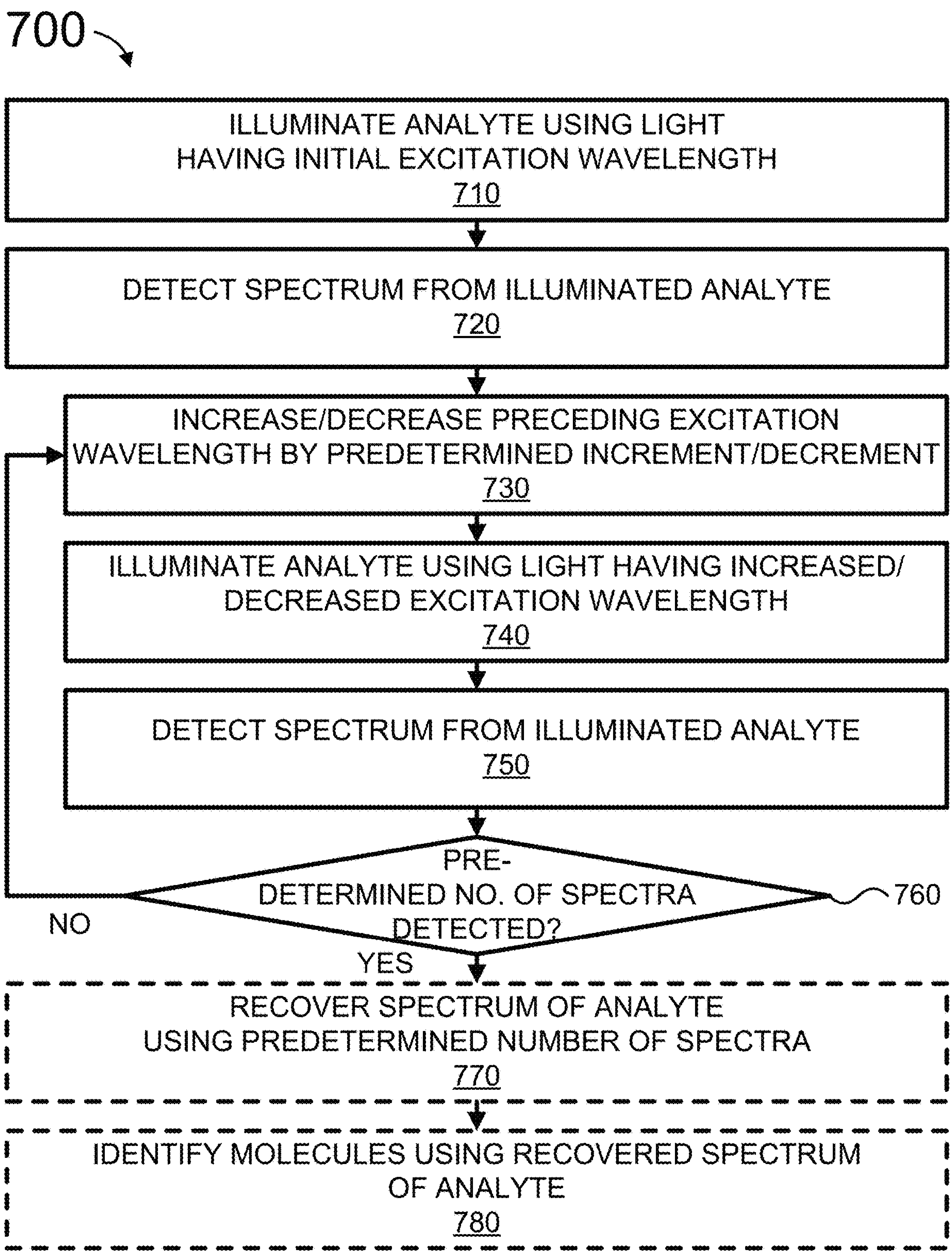

700
ILLUMINATE ANALYTE USING LIGHT HAVING INITIAL EXCITATION WAVELENGTH
710
DETECT SPECTRUM FROM ILLUMINATED ANALYTE
720
INCREASE/DECREASE PRECEDING EXCITATION WAVELENGTH BY PREDETERMINED INCREMENT/DECREMENT
730
ILLUMINATE ANALYTE USING LIGHT HAVING INCREASED/ DECREASED EXCITATION WAVELENGTH
740
DETECT SPECTRUM FROM ILLUMINATED ANALYTE
750
PRE-DETERMINED NO. OF SPECTRA DETECTED?
760
NO
YES
RECOVER SPECTRUM OF ANALYTE USING PREDETERMINED NUMBER OF SPECTRA
770
IDENTIFY MOLECULES USING RECOVERED SPECTRUM OF ANALYTE
780

FIG. 7

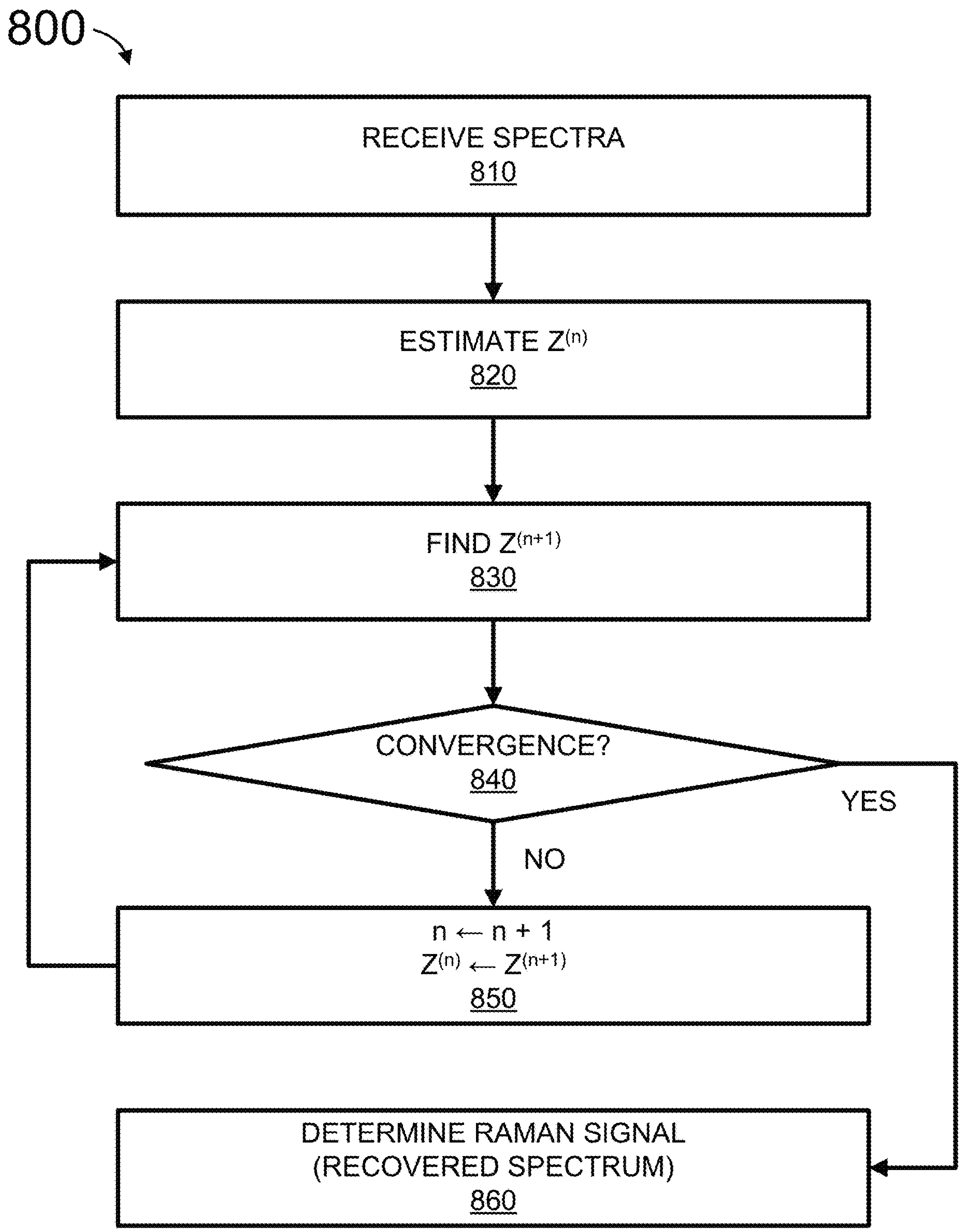

800
RECEIVE SPECTRA
810
ESTIMATE $Z^{(n)}$
820
FIND $Z^{(n+1)}$
830
CONVERGENCE?
840
YES
NO
$n \leftarrow n + 1$
$Z^{(n)} \leftarrow Z^{(n+1)}$
850
DETERMINE RAMAN SIGNAL
(RECOVERED SPECTRUM)
860

FIG. 8

900

910 920

MOLECULE	DIAGNOSTIC FOR
Carotenoid	Antioxidant levels
Glucose (HbA1c test)	Diabetes
Colon cancer biomarker (BM)	Cancer
Liver cancer BM	Cancer
Lung cancer BM	Cancer
Melanoma BM	Cancer
Stomach cancer BM	Cancer
HDL Cholestrol	Heart disease
LDL Cholestrol	Heart disease
Triglycerides	Heart disease

FIG. 9

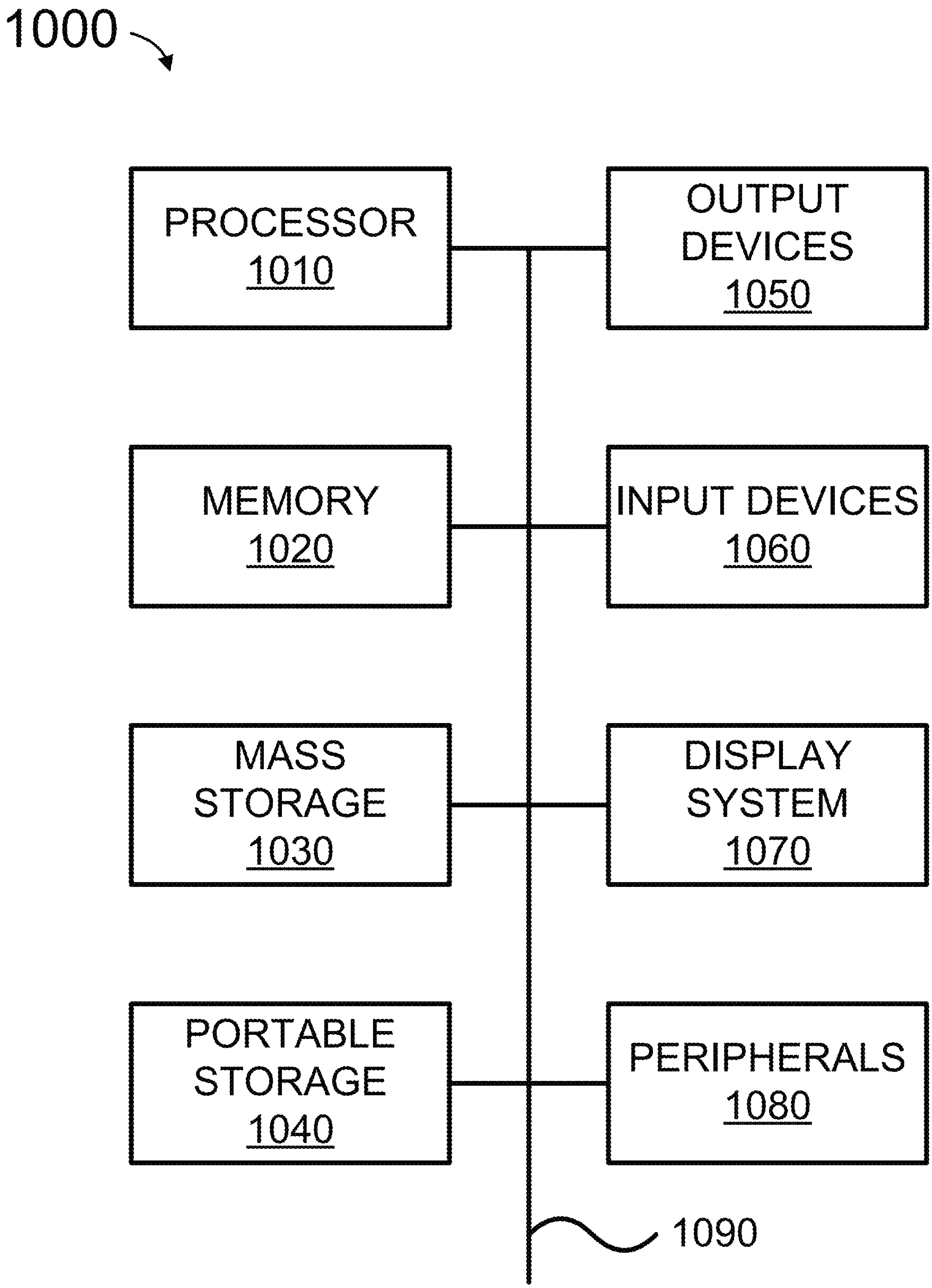

1000
PROCESSOR
1010
OUTPUT DEVICES
1050
MEMORY
1020
INPUT DEVICES
1060
MASS STORAGE
1030
DISPLAY SYSTEM
1070
PORTABLE STORAGE
1040
PERIPHERALS
1080
1090

FIG. 10

NON-INVASIVE MEASUREMENT OF BIOLOGICAL ANALYTES

TECHNICAL FIELD

The present technology relates generally to spectral imaging, and more specifically to measurement of biological analytes.

BACKGROUND

The approaches described in this section could be pursued but are not necessarily approaches that have previously been conceived or pursued. Therefore, unless otherwise indicated, it should not be assumed that any of the approaches described in this section qualify as prior art merely by virtue of their inclusion in this section.

Spectroscopy (or spectrography) refers to techniques that employ radiation in order to obtain data on the structure and properties of matter. Spectroscopy involves measuring and interpreting spectra that arise from the interaction of electromagnetic radiation (e.g., a form of energy propagated in the form of electromagnetic waves) with matter. Spectroscopy is concerned with the absorption, emission, or scattering of electromagnetic radiation by atoms or molecules.

Spectroscopy can include shining a beam of electromagnetic radiation onto a desired sample in order to observe how it responds to such stimulus. The response can be recorded as a function of radiation wavelength, and a plot of such responses can represent a spectrum. The energy of light (e.g., from low-energy radio waves to high-energy gamma-rays) can result in producing a spectrum.

SUMMARY

This summary is provided to introduce a selection of concepts in a simplified form that are further described in the Detailed Description below. This summary is not intended to identify key features or essential features of the claimed subject matter, nor is it intended to be used as an aid in determining the scope of the claimed subject matter.

The present disclosure is related to various systems and methods for non-invasive measurement of biological analytes. Specifically, a method for non-invasive measurement of biological analytes may comprise: illuminating an analyte using first light, the first light having a first excitation wavelength; detecting a first spectrum from the analyte illuminated by the first light, the first spectrum including a first Raman signal and fluorescence; illuminating the analyte using second light, the second light having a second excitation wavelength, the second excitation wavelength being larger than the first excitation wavelength by a first predetermined increment; detecting a second spectrum from the analyte illuminated by the second light, the second spectrum including a second Raman signal and the fluorescence, the second Raman signal being shifted from the first Raman signal by a second predetermined increment; illuminating the analyte using third light, the third light having a third excitation wavelength, the third excitation wavelength being larger than the second excitation wavelength by the first predetermined increment; detecting a third spectrum from the analyte illuminated by the third light, the third spectrum including a third Raman signal and the fluorescence, the third Raman signal being shifted from the second Raman signal by the second predetermined increment; recovering the first Raman signal using the first spectrum, the second spectrum, and the third spectrum using an inverse transform; and using the first Raman signal to identify and measure at least one molecule of the analyte using a database of identified Raman signals.

BRIEF DESCRIPTION OF THE DRAWINGS

Embodiments are illustrated by way of example, and not by limitation, in the figures of the accompanying drawings, in which like references indicate similar elements and in which:

FIG. **1** is a simplified representation of a system for non-invasive measurement of biological analytes, according to some embodiments.

FIG. **2** is a simplified representation of a system for non-invasive measurement of biological analytes, according to various embodiments.

FIG. **3** is a cross-sectional view of the system of FIG. **2**, in accordance with some embodiments.

FIGS. **4**A and **4**B are graphical representations of penetration depth into liquid water and absorption spectra of biological tissues, respectively, in accordance with various embodiments.

FIG. **5** is a simplified graphical representation of intensity, according to some embodiments.

FIG. **6** is a simplified graphical representation of intensity for more than one excitation wavelength, according to various embodiments.

FIG. **7** is a simplified flow diagram of a method for non-invasive measurement of biological analytes, in accordance with some embodiments.

FIG. **8** is a simplified flow diagram of a method for recovering a Raman spectrum, in accordance with various embodiments.

FIG. **9** is a table of molecules, according to some embodiments.

FIG. **10** is a simplified block diagram of a computing system, according to various embodiments.

DETAILED DESCRIPTION

While this technology is susceptible of embodiment in many different forms, there is shown in the drawings and will herein be described in detail several specific embodiments with the understanding that the present disclosure is to be considered as an exemplification of the principles of the technology and is not intended to limit the technology to the embodiments illustrated. The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the technology. As used herein, the singular forms "a," "an," and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise. It will be further understood that the terms "comprises," "comprising," "includes," and/or "including," when used in this specification, specify the presence of stated features, integers, steps, operations, elements, and/or components, but do not preclude the presence or addition of one or more other features, integers, steps, operations, elements, components, and/or groups thereof. It will be understood that like or analogous elements and/or components, referred to herein, may be identified throughout the drawings with like reference characters. It will be further understood that several of the figures are merely schematic representations of the present technology. As such, some of the components may have been distorted from their actual scale for pictorial clarity.

FIG. **1** illustrates system **100** for non-invasive measurement of biological analytes according to some embodiments. System **100** can include Raman instrument **110**A and analyte **150**A.

According to some embodiments, analyte **150**A is at least one of plant, human, and animal tissue. For example, animal tissue is one or more of epithelial, nerve, connective, muscle, and vascular tissues. By way of further non-limiting example, plant tissue is one or more of meristematic (e.g., apical meristem and cambium), protective (e.g., epidermis and cork), fundamental (e.g., parenchyma, collenchyma and sclerenchyma), and vascular (e.g., xylem and phloem) tissues.

According to some embodiments, Raman instrument **110**A comprises excitation light source **120**, detector **130**, and optionally sampling apparatus **140**. Excitation light source **120** is a monochromatic light source, such as a laser, in accordance with some embodiments. For example, excitation light source **120** is at least one of an Nd:YAG (neodymium-doped yttrium aluminium garnet; Nd:Y3Al5O12), Argon-ion, He—Ne, and diode laser. By way of further non-limiting example, excitation source **120** can provide light (electromagnetic waves) in a range between ultra-violet (UV) light (e.g., electromagnetic radiation with a wavelength from 10 nm to 400 nm) and short-wave near-infrared (NIR) (1.4 μm to 3 μm), including portions of the electromagnetic spectrum in-between, such as visible light (e.g., 380 nm-760 nm) and NIR light (e.g., 0.75 μm to 1.4 μm).

In various embodiments, excitation light source **120** is tunable—a wavelength of the light from excitation light source **120** is changed by one or more (predetermined) increments and/or to one or more (predetermined) values—such as by using heat control (e.g., from a heating element), electrical control (e.g., using microelectromechanical systems (MEMS)), and mechanical control (e.g., using a mechanism to turn a mirror). Preferably, excitation light source **120** provides high spectral purity, high wavelength stability, and/or high power stability output.

Optional sampling apparatus **140** performs various combinations and permutations of directing light **160**A from excitation light source **120**, collecting the resulting Raman scatter (among others) **170**A, filtering out radiation at the wavelength corresponding to the laser line (e.g., Rayleigh scattering), and providing the Raman scatter (among others) **170**A to detector **130**, according to some embodiments. For example, sampling apparatus **140** includes a microscope and/or an optical probe. By way of further non-limiting example, sampling apparatus **140** includes one or more filters (e.g., notch filter, edge-pass filter, and band-pass filter). Raman scatter (among others) **170**A includes, for example, at least one of Raman scatter, fluorescence, and Rayleigh scattering (which can be filtered out by sampling apparatus **140**).

In accordance with some embodiments, detector **130** is a spectrograph. For example, detector **130** includes slit **132**, spectral dispersion element **134**, and detector **136**. By way of non-limiting example, detector **130** measures wavelengths in one or more of the UV spectrum (10 nm to 400 nm), visible spectrum (e.g., 380 nm-760 nm), visible to near-infrared (e.g., 400 nm-1000 nm), short-wave infrared (e.g., 950 nm-1700 nm), and infrared (e.g., 1 μm-5 μm).

Slit **132** can determine the amount of light (e.g., photon flux, such as Raman scatter (among others) **170**A) that enters optical bench **138**. Dimensions (e.g., height and width, not shown in FIG. **1**) of slit **132** can determine the spectral resolution of detector **130**. By way of non-limiting example, a height of slit **132** can range from 1 mm to 20 mm. By way of further non-limiting example, a width of slit **132** can range from 5 μm to 800 μm.

Spectral dispersion element **134** can determine a wavelength range of detector **130** and can partially determine an optical resolution of detector **130**. For example, spectral dispersion element **134** is a ruled diffraction grating or a holographic diffraction grating, in the form of a reflective or transmission package. Spectral dispersion element **134** can include a groove frequency and a blaze angle.

Detector **136** receives light and measures the intensity of scattered light. Detector **136** can be a one- or two-dimensional detector array comprised of a semiconductor material such as silicon (Si) and indium gallium arsenide (InGaAs). In some embodiments, a bandgap energy of the semiconductor determines an upper wavelength limit of detector **136**. An array of detector **136** can be in different configurations, such as charged coupled devices (CCDs), back-thinned charge coupled devices (BT-CCDs), complementary metal-oxide-semiconductor (CMOS) devices, and photodiode arrays (PDAs). CCDs can be one or more of intensified CCDs (ICCDs) with photocathodes, back illuminated CCDs, and CCDs with light enhancing coatings (e.g., Lumogen® from BASF®). Detector **136** has a resolution of 8-15 wavenumbers, according to some embodiments. Detector **136** can be used to detect concentrations of molecules in the range of 1-1,000 mg per deciliter (mg/dL).

Optical bench **138** of detector **130** includes slit **132**, spectral dispersion element **134**, detector **136**, and various optical elements (not shown in FIG. **1**). Slit **132**, spectral dispersion element **134**, and detector **136** can be arranged in optical bench **138**, along with other components (e.g., monochromater—which transmits a mechanically selectable narrow band of wavelengths of light or other radiation chosen from a wider range of wavelengths available at an input—including one or more of a mirror, prism, collimater, holographic grating, diffraction grating, blazed grating, and the like), according to different configurations. For example, different configurations include: crossed Czerny-Turner, unfolded Czerny-Turner, transmission, and concave holographic optical benches.

Raman instrument **110**A can provide information about molecular vibrations to identify and quantify characteristics (e.g., molecules) of analyte **150**A. Raman instrument **110**A can direct light (electromagnetic waves) **160**A from excitation source **120** (optionally through sampling apparatus **140**) onto analyte **150**A. Light **160**A from excitation source **120** can be said to be shone on analyte **150**A and/or analyte **150**A can be said to be illuminated by excitation source **120** and/or light **160**A. When (incident) light from excitation source **120** hits analyte **150**A, the (incident) light scatters. A majority (e.g., 99.999999%) of the scattered light is the same frequency as the light from excitation source **120** (e.g., Rayleigh or elastic scattering).

A small amount of the scattered light (e.g., on the order of 10^{-6} to 10^{-8} of the intensity of the (incident) light from excitation source **120**) is shifted in energy from the frequency of light **160**A from excitation source **120**. The shift is due to interactions between (incident) light **160**A from excitation source **120** and the vibrational energy levels of molecules in analyte **150**A. (Incident) Light **160**A interacts with molecular vibrations, phonons, or other excitations in analyte **150**A, causing the energy of the photons (of light **160**A from excitation source **120**) to shift up or down (e.g., Raman or inelastic scattering). The shift in energy (e.g., of light **170**A from analyte **150**A) can be used to identify and quantify characteristics (e.g., molecules) of analyte **150**A.

Detector **130** detects (an intensity of) the Raman scattering using detector **136** (optionally received through sampling apparatus **140**). A Raman spectrograph—a plot/graph of an intensity of the Raman scattering (shifted light) against frequency—can be produced by a computing system (not shown in FIG. **1**) using intensity measurements from detector **130**. The computing system can be integrated in or external to Raman instrument **110**A. The Raman spectrograph can reliably be used to identify molecules in analyte **150**A. In this way, a Raman spectrograph can be said to produce a "fingerprint" of molecules in analyte **150**A. For example, a Raman spectrograph of analyte **150**A can be compared to a database (e.g., in the same or another computing system) of Raman spectrographs associated with known molecules to identify and quantify molecules in analyte **150**A.

According to some embodiments, Raman instrument **110**A offers at least some of the advantages of: differentiating chemical structures (even if they contain the same atoms in different arrangements), physical contact with analyte **150**A not required, no damage to analyte **150**A (e.g., non-destructive testing), preparation of analyte **150**A is not required, analyte **150**A can be in a transparent container (e.g., when light **160**A is in the visible or near-visible light spectrum), sensitivity to small changes in material structure (e.g., detection of molecular vibrations is very sensitive to changes in chemistry and structure), analyzing samples in aqueous solutions (e.g., suspensions, biological samples, etc.), and the like.

FIG. **2** illustrates system **200** for non-invasive measurement of biological analytes according to various embodiments. System **200** includes Raman instrument **110**B and analyte **150**B. Analyte **150**B has at least some of the characteristics of analyte **150**A (FIG. **1**). Raman instrument **110**B is depicted as being directed to a surface **250**A of analyte **150**B purely for illustrative purposes. Raman instrument **110**B can be oriented toward other surfaces of analyte **150**B, such as surface **250**B. Moreover, analyte **150**B is depicted as a (human) finger purely for illustrative purposes. Other plant or animal tissue can be used. Alternatively or additionally, other parts of a human body (e.g., including a blood vessel, such as an earlobe, neck, face, back, chest, arm, leg, toe, and the like) may be used.

Raman instrument **110**B has at least some of the characteristics of Raman instrument **110**A (FIG. **1**). Raman instrument **110**B can include aperture **210**A. Aperture **210**A can be an opening through which light **160**A from excitation source **120** (FIG. **1**) exits Raman instrument **110**B and/or through which Raman scatter (among others) **170**A enters Raman instrument **110**B. For example, analyte **150**B is illuminated by excitation source **120** through aperture **210**A and the Raman scatter (among others) **170**A (FIG. **1**) from analyte **150**B is received by detector **130** (FIG. **1**) through aperture **210**A. Aperture **210**A can include at least some of the features of sampling apparatus **140** (FIG. **1**). Although aperture **210**A is shown as one opening, aperture **210**A can be more than one opening.

Raman instrument **110**B can optionally include surface **220**. In some embodiments, surface **220** is a surface on which analyte **150**B is placed so that analyte **150**B is positioned for measurement by Raman instrument **110**B and/or analyte **150**B does not substantially move during operation of Raman instrument **110**B (e.g., substantial movement would cause a sample to change between measurements).

Raman instrument **110**B can be a portable, handheld, or compact unit which can operate on battery power. Raman instrument **110**B can be communicatively coupled to computing system **240** through communications **230**. Communications **230** can be various combinations and permutations of wired and wireless communications (e.g., networks) described below in relation to FIG. **10**. Computing system **240** can include a database of Raman spectrographs associated with known molecules and/or remotely access the database over a communications network (not shown in FIG. **2**). In some embodiments, computing system receives intensity measurements from Raman instrument **110**B, produces at least one Raman spectrograph using data (e.g., intensity measurements) from Raman instrument **110**B, and identifies and/or quantifies molecules in analyte **150**B using the at least one Raman spectrograph and a database of Raman spectrographs associated with known molecules. Computing system **240** is described further below in relation to FIG. **10**.

In some embodiments, computing system **240** is a single computing device. For example, computing system **240** is a desktop or notebook computer communicatively coupled to Raman instrument **110**B through a Universal Serial Bus (USB) connection, a WiFi connection, and the like.

In various embodiments, computing system **240** is more than one (physical) computing device. For example, computing system **240** is a smart phone and a cloud-based computing system. The smart phone can receive data (e.g., intensity measurements) from Raman instrument **110**B using USB, WiFi, Blutooth, and the like. The smart phone can optionally produce at least one Raman spectrum (e.g., including the Raman signal and fluorescence, for each excitation wavelength) using the data. The smart phone can transmit the data and/or at least one Raman spectrum to a cloud-based computing system over the Internet using a wireless network (e.g., cellular network). The cloud-based computing system can produce at least one Raman spectrum using the data, recover a Raman spectrograph (e.g., without fluorescence) from the at least one received/produced Raman spectrum, and/or quantify and/or identify molecules in analyte **150**B using the recovered Raman spectrograph.

By way of further non-limiting example, communications **230** and at least some of computing system **240** can be in a dock (or cradle or pad) (not depicted in FIG. **2**) in (or on or adjacent to) which Raman instrument **110**E is placed. When Raman instrument **110**B is placed in (or on or adjacent to) the dock, communications **230** between Raman instrument **110**B and computing system **240** can be various combinations and permutations of wired and/or wireless communications. Alternatively or additionally, the dock can charge a rechargeable battery (e.g., lithium ion battery) of Raman instrument **110**B using wired and/or wireless charging. For example, the dock can include a connector (or plug or socket or other electrical contacts) which mates with a connector (or socket or plug or other electrical contacts) of Raman instrument **110**B (not depicted in FIG. **2**) for communications and/or charging. By way of further non-limiting example, the dock (and Raman instrument **110**B) can include at least one antenna, coil, and the like for wireless communications and/or charging. Other combinations and permutations of communications **230** and computing system **240** (e.g., as described below in relation to FIG. **10**) may be used.

FIG. **3** shows system **300**, which is a simplified cross-sectional view of system **200** (FIG. **2**) for non-invasive measurement of biological analytes, in accordance with some embodiments. System **300** includes Raman instrument **110**C and analyte **150**C. Raman instrument **110**C has at least some of the characteristics of Raman instrument **110**A (FIG.

1) and Raman instrument **110**B (FIG. **2**). Raman instrument **110**C can include aperture **210**B has at least some of the characteristics of aperture **210**A (FIG. **2**). Analyte **150**C has at least some of the characteristics of analyte **150**A (FIG. **1**) and analyte **150**B (FIG. **2**).

Analyte **150**C can include layers, such as epidermis **310**, dermis **330**, and subcutaneous (fatty) tissue **340**. Dermis **330** includes blood vessel **320** (e.g., vein and/or artery). For pictorial clarity, some features of epidermis **310**, dermis **330**, and subcutaneous (fatty) tissue **340** (e.g., hair shaft, sweat pore and duct, sensory nerve ending, sebaceous gland, pressure sensor, hair follicle, stratum, and the like) are not shown in FIG. **3**.

Light **160**B can have at least some of the characteristics of light **160**A (FIG. **1**). Light **160**B (e.g., from excitation light source **120** (FIG. **1**)) illuminates analyte **150**C through aperture **210**B. Light **160**B can pass through epidermis **310** to dermis **330**. Photons of light **160**B can bounce off molecules inside blood vessel **320**. (Resulting) Raman scatter (among others) **170**B is received by detector **130** (FIG. **1**) through aperture **210**B. Raman scatter (among others) **170**B can have at least some of the characteristics of Raman scatter (among others) **170**A (FIG. **1**).

FIG. **4**A is a graphical representation (e.g., plot, graph, and the like) **400**A of penetration depth **410**A into liquid water of light over excitation wavelength. By way of non-limiting example, an epidermis (e.g., epidermis **310** in FIG. **3**) can have a thickness on the order of 100 μm, so an excitation wavelength of light (e.g., light **160**A and light **160**B in FIGS. **1** and **3**, respectfully) can be advantageously selected such that a penetration depth is at least 100 μm (e.g., approximately 190 nm to 2400 nm). In some embodiments, the excitation wavelength of light is in a range of 670 nm-900 nm for (human) tissue. Other ranges for the excitation wavelength of light can be used (e.g., depending on the depth of the tissue to be studied).

FIG. **4**B is a graphical representation (e.g., plot, graph, and the like) **400**B of absorption spectra of various tissues over excitation wavelength. By way of non-limiting example, an excitation wavelength of light (e.g., light **160**A and light **160**B in FIGS. **1** and **3**, respectfully) can be advantageously selected to minimize the absorption coefficient so as to minimize absorption of the light by the tissue to be studied (e.g., so the light can scatter and be detected). When the tissue substantially absorbs light and/or Raman scatter (among others) (e.g., **170**A and **170**B in FIGS. **1** and **3**, respectively), there can be insufficient electromagnetic radiation for detector **130** to detect. In various embodiments, the excitation wavelength of light is in a range of 670 nm-900 nm for (human) tissue. Other ranges for the excitation wavelength of light can be used (e.g., depending on the absorption coefficient of the tissue to be studied).

In embodiments where analyte (e.g., **150**A-C (FIGS. **1**-**3**)) is a live (and not dead) animal (e.g., living, alive, etc.), blood flows through blood vessel **320** (FIG. **3**). Blood flow through blood vessel **320** in animals (e.g., humans) is caused by a heart (not shown in FIG. **4**) pumping blood (e.g., beating heart). When measurements are taken at a rate slower than blood flows, different samples of blood are measured instead of the same sample and fluorescence will change with each sample.

When Raman instrument **110**C takes multiple measurements (as described below in relation to FIGS. **6** and **7**), the measurements can be taken before the molecules in blood illuminated in one measurement (e.g., blood sample) flow away and are not available for the next measurement. For example, a resting adult human heart can beat at approximately 60 to 100 beats a minute (~1 Hz). Raman instrument **110**C can take measurements within a tenth of a second (~0.1 KHz) or less, such that measurements are taken faster than blood flows (e.g., multiple measurements are taken from the same (instead of different) sample). Slower and/or faster sampling rates (e.g., frequency at which measurements are taken) can be used depending on the heart rate associated with analyte **150**C (FIG. **3**). In various embodiments, the sampling rate is 10 Hz -1 KHz.

FIG. **5** is a graphical representation (e.g., plot, graph, and the like) **500** of received light intensity (in units mW) (along axis **520**) over received light wavelength (along axis **510**) in nm. Graph **500** includes Raman signal **530** (**530**A-**530**D) and fluorescence **540**, according to some embodiments. Raman signal **530** is a Raman spectrograph for an analyte (e.g., analyte **150**A-C (FIGS. **1**-**3**) that would be measured if it were not overwhelmed/obscured by fluorescence **540**. Although Raman signal **530** is shown having four peaks at regular intervals, Raman signal **530** may have any number of peaks having different intensities and occurring at different/irregular frequencies. The peaks of Raman signal **530** can indicate information about different molecular bonds.

When light (e.g., light **160**A and **160**B in FIGS. **1** and **3**, respectively) illuminates analyte (e.g., analyte **150**A-C in FIGS. **1**-**3**, respectively), fluorescence **540** (in addition to Raman signal **530**) can result. Fluorescence **540** can be several orders of magnitude (e.g. 10^5-10^6) higher in intensity than Raman signal **530**. Fluorescence **540** can overwhelm or obscure Raman signal **530**, such that Raman signal **530** is difficult to actually measure.

An intensity measured by detector **130** (FIG. **1**) includes an intensity (I) of the Raman signal (IR) and intensity of fluorescence (IF) at each wavelength (e.g., $I=I_R+I_F$). For example, the intensity measured by detector **130** (FIG. **1**) would look like fluorescence **540** with very small contributions **550**A-**550**D from Raman signal **530** (**530**A-**530**D). Contributions **550**A-**550**D are provided for illustrative purposes and are not drawn to scale. Flourescence **540** is several orders of magnitude (e.g. 10^5-10^6) larger than Raman signal **530** and contributions **550**A-**550**D and may not be visible if shown to scale.

An intensity of the Raman signal is inversely proportional to the excitation wavelength (λ) of light (e.g., light **160**A and **160**B in FIGS. **1** and **3**, respectively) (e.g., Raman signal strength $\alpha\lambda^{-4}$). In contrast, an intensity of the fluorescence is proportional to the excitation wavelength (λ). Generally, when a longer excitation wavelength (λ) is used to illuminate tissue, there is less fluorescence but the Raman signal strength becomes smaller and difficult to measure. Likewise, when a shorter excitation wavelength (λ) is used (e.g., in the near infrared (NR) spectrum) to illuminate tissue, too much fluorescence is produced making it difficult to measure the Raman signal.

FIG. **6** is a graphical representation (e.g., plot, graph, and the like) **600** of received light intensity (in mW) (along axis **520**) over received light wavelength in nm (along axis **510**), according to some embodiments. Graph **600** includes Raman signal **530** (**530**A-**530**D), Raman signal **610** (**610**A-**610**D), Raman signal **620** (**620**A-**620**D), and fluorescence **540**. Raman signal **530** and fluorescence **540** were described above in relation to FIG. **5**. Raman signals **610** and **620** are Raman spectrographs for analyte **150**C (FIG. **3**) that would be measured if it were not overwhelmed/obscured by fluorescence **540**. Although Raman signals **610** and **620** are shown each having four peaks at regular intervals, Raman signals **610** and **620** may have any number of peaks having different intensities and occurring at different/irregular frequencies (e.g., corresponding to or following Raman signal **530**). Raman signals **530**, **610**, and **620** can result from different excitation wavelengths (λ).

As described above, excitation light source **120** (FIG. **1**) can be tunable, such that an excitation wavelength can change (e.g., by a predetermined increment, to one or more predetermined wavelengths, etc.). When measurements are (sequentially) taken at different excitation wavelength (λ) (e.g., $\lambda=\lambda_0, \lambda_1, \lambda_2, \ldots$), a Raman signal for each excitation wavelength can be produced. For example, Raman signal **530** (**530**A-**530**D) is measured at $\lambda=\lambda_0$, Raman signal **610** (**610**A-**610**D) at $\lambda=\lambda_1$, and Raman signal **620** (**620**A-**620**D) $\lambda=\lambda_2$. Although three different excitation wavelengths (e.g., $\lambda=\lambda_0, \lambda_1, \lambda_2$) are used, any number N of different excitation wavelengths can be used (e.g., $\lambda=\lambda_0, \lambda_1, \ldots \lambda_N$). N can be a function of a sampling rate of Raman instrument (e.g., Raman instrument **110**A (FIG. **1**), **110**B (FIGS. **2**), and **110**C (FIG. **3**)), a molecule to be detected and/or quantified, and the like. The excitation wavelength can be incremented by a predetermined amount $\Delta\lambda$, such that $\lambda_1=\lambda_0+\Delta\lambda$, $\lambda_2=\lambda_1+\Delta\lambda$, $\lambda_3=\lambda_2+\Delta\lambda$, etc. As shown in FIG. **6**, Raman signals **610** and **620** can be shifted from an adjacent Raman signal (e.g., Raman signals **530** and **610**, respectively) by $\Delta\lambda$. Although Raman signals **530**, **610**, and **620** are shifted (e.g., by $\Delta\lambda$), the envelopes (e.g., amplitude and frequency of the peaks) of Raman signals **530**, **610**, and **620** are consistent. At each of $\lambda=\lambda_0, \lambda_1, \lambda_2, \ldots$, fluorescence **540** is the same (e.g., as long as the (blood) sample does not change).

An intensity measured by detector **130** (FIG. **1**) includes an intensity (I) of the Raman signal (I_R) and intensity of fluorescence (I_F) at each wavelength (e.g., $I=I_R+I_F$), as described above in relation to FIG. **5**. For example, for excitation wavelength $\lambda=\lambda_1$, the Raman spectrograph would look like fluorescence **540** with very small contributions (e.g., contributions **630**A-D) from Raman signal **610** (**610**A-**610**D). By way of further non-limiting example, for excitation wavelength $\lambda=\lambda_2$, the Raman spectrograph would look like fluorescence **540** with very small contributions (e.g., **640**A-D) from Raman signal **620** (**620**A-**620**D). Contributions **630**A-D and **640**A-D are provided for illustrative purposes and are not drawn to scale.

As described below in relation to FIGS. **7** and **8**, a Raman spectrograph for analyte **150**C (e.g., compensating for fluorscence) can be produced using Raman signals **530** (**530**A-**530**D), **610** (**610**A-**610**D), **620** (**620**A-**620**D), etc.

FIG. **7** illustrates a method **700** for non-invasive measurement of biological analytes, according to some embodiments. Method **700** can he performed by a Raman instrument and/or a computing system. The Raman instrument can have at least some of the characteristics of Raman instrument **110**A (FIG. **1**), Raman instrument **110**B (FIG. **2**), and Raman instrument **1100** (FIG. **3**). The computing system can have at least some of the characteristics of computing system **240** (FIG. **2**) and computing system **1000** (FIG. **10**).

Method **700** can commence at step **710**, where an analyte can be illuminated using light having an initial excitation wavelength. For example, the analyte has at least some of the characteristics of analyte **150**A (FIG. **1**), analyte **150**B (FIG. **2**), and analyte **150**C (FIG. **3**). By way of further non-limiting example, the light can be provided by the Raman instrument, for example, using excitation light source **120** (FIG. **1**). For illustrative purposes, the initial excitation wavelength can referred to as λ_0 and can have a value of 670 nm (e.g., λ_0=670 nm). Other values for λ_0 can be used.

At step **720**, a spectrum (e.g., including Raman scattering (or Raman signal) and fluorescence) can be detected from the illuminated analyte. In some embodiments, the light hitting the analyte results in Raman scattering (or Raman signal) and fluorescence. For example, the Raman scattering (e.g., contributions **550**A-D, **630**A-D, and **640**A-D) and fluorescence (e.g., fluorescence **540**) can be detected by the Raman instrument (e.g., using detector **130** optionally through sampling apparatus **140** (FIG. **1**)). By way of further non-limiting example, the detected Raman scattering (e.g., contributions **550**A-D) and fluorescence (e.g., fluorescence **540**) may appear (e.g., when graphed, plotted, and the like) as shown in graphical representation **500** (FIG. **5**) (where the excitation wavelength is λ_0). The detected spectrum (e.g., data, graphical representation, and the like) can be stored by (and/or in) the Raman instrument and/or the computing system.

At step **730**, the preceding excitation wavelength can be increased or decreased by a predetermined increment or decrement, respectively. For illustrative purposes, the predetermined increment/decrement can be referred to as $\Delta\lambda$. For example, when the preceding excitation wavelength is λ_0, an increased/decreased excitation wavelength is λ_1, where $\lambda_1=\lambda_0+\Delta\lambda$. By way of further non-limiting example, when the preceding excitation wavelength is λ_1, an increased/decreased excitation wavelength is λ_2, where $\lambda_2=\lambda_1+\Delta\lambda$. By way of additional non-limiting example, when N spectra are to be detected, $\Delta\lambda=\lambda_0+(A*\Delta\lambda)$, where $A=\{0, 1, \ldots (N-1)\}$.

For illustrative purposes, the predetermined increment/decrement can have a value of 0.5 nm. To illustrate embodiments where the excitation wavelength is increased, when λ_0=670 nm, λ_1=670.5 nm, λ_2=671 nm, and so on according to the number of spectra to be detected (N). In some embodiments, the excitation wavelength is decreased by a decrement.

At step **740**, the analyte can be illuminated using light having the increased or decreased wavelength. To illustrate embodiments where the excitation wavelength is increased, the light can have a wavelength λ_1=670.5 nm, λ_2=671 nm, or so on according to the number of spectra to be detected (N).

At step **750**, a spectrum (e.g., including Raman scattering (or Raman signal) and fluorescence) can be detected from the illuminated analyte. In some embodiments, the light (having the increased/decreased excitation wavelength) hitting the analyte results in Raman scattering (or Raman signal) and fluorescence. For example, the Raman scattering and fluorescence can be detected by the Raman instrument (e.g., using detector **130** optionally through sampling apparatus **140** (FIG. **1**)). The detected Raman scattering and fluorescence may appear (e.g., when graphed/plotted) as shown in graph **500** (FIG. **5**) (where the excitation wavelength is the the increased/decreased excitation wavelength, for example, λ_1, λ_2, and so on according to the number of spectra to be detected). Each detected spectrum (e.g., data, graphical representation, and the like) can be stored by (and/or in) the Raman instrument and/or the computing system.

At step **760**, a determination is made as to whether another spectrum is to be detected. In some embodiments, the predetermined number of spectra to be detected (N) is compared to the number of spectra (actually) detected. When the predetermined number of spectra to be detected (N) is less than the number of spectra detected, method **700** can proceed to step **730**. When the predetermined number of spectra to be detected (N) is equal to the number of spectra actually detected, method **700** can proceed to step **770**. For example, when N=6 and spectra are already detected for λ_0, λ_1, λ_2, λ_3, λ_4, and λ_5, method **700** can proceed to step **770**. By way of further non-limiting example, when N=**3** the detected Raman scattering and fluorescence (e.g., detected for each of λ_0, λ_1, and λ_2) may appear (e.g., when graphed/plotted together) as shown in graph **600** (FIG. **6**).

Optionally at step **770**, a Raman spectrum of the analyte can be recovered using the detected spectra (e.g., N detected spectra). In some embodiments, the Raman spectrum of the analyte can be recovered using expectation maximization techniques. The recovered Raman spectrum may appear (e.g., when graphed/plotted) as shown in graph **500** (FIG. **5**) (e.g., Raman signal **530** (**530**A-D) without fluorescence **540**). Recovering the Raman spectrum of the analyte is described further below in relation to FIG. **8**.

Optionally at step **780**, a molecule can be identified using the recovered Raman spectrum. For example, a database of known Raman spectrum for certain molecules can be searched using (e.g., compared to) the recovered Raman spectrum to find a match.

FIG. **8** shows a method **800** for recovering a Raman spectrum of an analyte using expectation maximization techniques and the detected spectra, according to some embodiments. Method **800** can commence at step **810**, where the detected spectra (e.g., N detected spectra) can be received. By way of non-limiting example, the detected spectra are referred to as vector X. The detected intensity in vector X includes the intensity of fluorescence and the Raman signal (e.g., $I=I_R+I_F$). According to some embodiments, vector X (e.g., detected spectra) can be represented by:

$$X = \begin{bmatrix} Y_{1,1} \\ Y_{1,2} \\ \vdots \\ Y_{1,N} \\ Y_{2,1} \\ Y_{2,2} \\ \vdots \\ Y_{2,N} \\ \vdots \\ Y_{K-1,N} \\ Y_{K,1} \\ Y_{K,2} \\ \vdots \\ Y_{K,N} \end{bmatrix} \tag{1}$$

where each Y_i (where i={1, 2, . . . K}) is a measured spectrum using a different excitation wavelength.

By way of further non-limiting example, the (separate) values of the fluorescence and the Raman signal are referred to as vector Z. Vector Z (e.g., (separate) values of the fluorescence and the Raman signal) can be represented by a vector have 2N dimensions:

$$Z = \begin{bmatrix} S_1^F \\ S_2^F \\ \vdots \\ S_N^F \\ S_1^R \\ S_2^R \\ \vdots \\ S_N^R \end{bmatrix} \tag{2}$$

where the fluorescence spectrum is S^F and the Raman spectrum is S^R.

A relationship between vector X and vector Z can be represented as a matrix of (predetermined) parameters, matrix H. By way of non-limiting example, a relationship between vector X, vector Z, and matrix H can be:

$$H \times Z = X \tag{3}$$

where matrix H can be represented by a KN×2N matrix having predetermined values, such as:

$$H = \begin{bmatrix} 1, 0, 0, \ldots, 0 \\ 0, 1, 0, \ldots, 0 \\ \vdots \\ 0, 0, 0, \ldots, 1 \\ 0, 0, 0, \ldots, 0 \\ 1, 0, 0, \ldots, 0 \\ 0, 1, 0, \ldots, 0 \\ \vdots \\ 0, 0, 0, \ldots, 1 \end{bmatrix} \tag{4}$$

The relationship depicted in equation 3 is an inverse problem: using a known vector X to determine vector Z, where matrix H is a large matrix which cannot be inverted. In various embodiments, the inverse problem in equation 3 is solved using Maximum Likelihood-Expectation Maximization (ML-EM) iterative methods included in method **800**. For example, among all possible values for vector Z, one that maximizes the probability of producing vector X is selected. The maximization can be performed using the Expectation Maximization (EM) techniques included in method **800**.

At step **820**, an initial guess vector $Z^{(n=0)}$ can be used for vector Z (e.g., S^F and S^R). In some embodiments, vector $Z^{(n=0)}$ can be arbitrary, a prior calculated estimate of vector Z (e.g., using method **800**), combinations thereof, and the like.

At step **830**, an estimate for vector Z (e.g., $Z^{(n+1)}$) can be determined. For example, Z can be estimated using:

$$z_i^{(n+1)} = z_i^n * \left(\frac{1}{\sum_j H_{ji}}\right) * \left(\sum_j H_{ji}\right) * \left(\frac{X_j}{\sum_k H_{jk} Z_k^n}\right) \tag{5}$$

At step **840**, the estimate for vector Z (e.g., vector $Z^{(n+1)}$) can be evaluated. In some embodiments, the estimate for vector Z is evaluated for convergence. For example, when a change between successive iterations (e.g., between vector Z^n and vector Z^{n+k}, where k can be a number in the range of 0-10,000) is smaller than a predetermined amount (e.g., tolerance, such as 1%-10% change), then vector Z can be said to converge. The change can be determined between an iteration early in the method (e.g., vector Z^j (where j can be a number in the range of 5-10,000) and a latest iteration. Additionally or alternatively, vector Z can be said to have converged after a predetermined number (e.g., 10-50,000) of iterations. In various embodiments, for some spectra having different fluorescence levels, changes in the estimate for vector Z are negligible (e.g., smaller than a predetermined amount) after around 2,000 iterations (e.g., 1,000-3,000 iterations). When vector Z has not converged or immediately after the first iteration (e.g., using vector $Z^{(n=0)}$), method **800** can proceed to step **850**. When vector Z is determined to have converged, method **800** can proceed to step **860**.

At step **850**, n can be incremented (e.g., $n \leftarrow n+1$), Z can be incremented (e.g., $Z^{(n)} \leftarrow Z^{(n+1)}$) and method **800** can perform another iteration by proceeding to step **830**.

At step **860**, a next estimate for vector Z can be determined using vector X, matrix H, and the estimate for vector Z calculated in the prior iteration.

In various embodiments, method **800** can be performed multiple times, each repetition using a different initial guess $Z^{(n=0)}$. For example, the initial guesses can be various combinations and permutations of arbitrary, prior calculated estimate of Z (e.g., using method **800**), and the like. A vector Z can be selected from among the repetitions of method **800**.

FIG. **9** depicts a table **900** of example molecules **910** which may be detected by the systems (e.g., system **100** (FIG. **1**), system **200** (FIG. **2**), and system **200** (FIG. **2**)) and detected using methods (e.g., method **700** (FIG. **7**) and method **800** (FIG. **8**)) described herein. Conditions **920** associated with each molecule **910** are shown for illustrative purposes.

FIG. **10** illustrates an exemplary computer system **1000** that may be used to implement some embodiments of the present invention. The computer system **1000** in FIG. **10** may be implemented in the contexts of the likes of computing systems, networks, servers, or combinations thereof. The computer system **1000** in FIG. **10** includes one or more processor unit(s) **1010** and main memory **1020**. Main memory **1020** stores, in part, instructions and data for execution by processor unit(s) **1010**. Main memory **1020** stores the executable code when in operation, in this example. The computer system **1000** in FIG. **10** further includes a mass data storage **1030**, portable storage device **1040**, output devices **1050**, user input devices **1060**, a graphics display system **1070**, and peripheral device(s) **1080**.

The components shown in FIG. **10** are depicted as being connected via a single bus **1090**. The components may be connected through one or more data transport means. Processor unit(s) **1010** and main memory **1020** are connected via a local microprocessor bus, and the mass data storage **1030**, peripheral device(s) **1080**, portable storage device **1040**, and graphics display system **1070** are connected via one or more input/output (I/O) buses.

Mass data storage **1030**, which can be implemented with a magnetic disk drive, solid state drive, or an optical disk drive, is a non-volatile storage device for storing data and instructions for use by processor unit(s) **1010**. Mass data storage **1030** stores the system software for implementing embodiments of the present disclosure for purposes of loading that software into main memory **1020**.

Portable storage device **1040** operates in conjunction with a portable non-volatile storage medium, such as a flash drive, floppy disk, compact disk, digital video disc, or Universal Serial Bus (USB) storage device, to input and output data and code to and from the computer system **1000** in FIG. **10**. The system software for implementing embodiments of the present disclosure is stored on such a portable medium and input to the computer system **1000** via the portable storage device **1040**.

User input devices **1060** can provide a portion of a user interface. User input devices **1060** may include one or more microphones, an alphanumeric keypad, such as a keyboard, for inputting alphanumeric and other information, or a pointing device, such as a mouse, a trackball, stylus, or cursor direction keys. User input devices **1060** can also include a touchscreen. Additionally, the computer system **1000** as shown in FIG. **10** includes output devices **1050**. Suitable output devices **1050** include speakers, printers, network interfaces, and monitors.

Graphics display system **1070** include a liquid crystal display (LCD) or other suitable display device. Graphics display system **1070** is configurable to receive textual and graphical information and processes the information for output to the display device.

Peripheral device(s) **1080** may include any type of computer support device to add additional functionality to the computer system.

The components provided in the computer system **1000** in FIG. **10** are those typically found in computer systems that may be suitable for use with embodiments of the present disclosure and are intended to represent a broad category of such computer components that are well known in the art. Thus, the computer system **1000** in FIG. **10** can be a personal computer (PC), hand held computer system, telephone, mobile computer system, workstation, tablet, phablet, mobile phone, server, minicomputer, mainframe computer, wearable, or any other computer system. The computer may also include different bus configurations, networked platforms, multi-processor platforms, and the like. Various operating systems may be used including UNIX, LINUX, WINDOWS, MAC OS, PALM OS, QNX, ANDROID, IOS, CHROME, and other suitable operating systems.

Some of the above-described functions may be composed of instructions that are stored on storage media (e.g., computer-readable medium). The instructions may be retrieved and executed by the processor. Some examples of storage media are memory devices, tapes, disks, and the like. The instructions are operational when executed by the processor to direct the processor to operate in accord with the technology. Those skilled in the art are familiar with instructions, processor(s), and storage media.

In some embodiments, the computing system **1000** may be implemented as a cloud-based computing environment, such as a virtual machine operating within a computing cloud. In other embodiments, the computing system **1000** may itself include a cloud-based computing environment, where the functionalities of the computing system **1000** are executed in a distributed fashion. Thus, the computing system **1000**, when configured as a computing cloud, may include pluralities of computing devices in various forms, as will be described in greater detail below.

In general, a cloud-based computing environment is a resource that typically combines the computational power of a large grouping of processors (such as within web servers) and/or that combines the storage capacity of a large grouping of computer memories or storage devices. Systems that provide cloud-based resources may be utilized exclusively by their owners or such systems may be accessible to outside users who deploy applications within the computing infrastructure to obtain the benefit of large computational or storage resources.

The cloud is formed, for example, by a network of web servers that comprise a plurality of computing devices, such as the computing system **1000**, with each server (or at least a plurality thereof) providing processor and/or storage resources. These servers manage workloads provided by multiple users (e.g., cloud resource customers or other users). Typically, each user places workload demands upon the cloud that vary in real-time, sometimes dramatically. The nature and extent of these variations typically depends on the type of business associated with the user.

It is noteworthy that any hardware platform suitable for performing the processing described herein is suitable for use with the technology. The terms "computer-readable storage medium" and "computer-readable storage media" as used herein refer to any medium or media that participate in providing instructions to a CPU for execution. Such media can take many forms, including, but not limited to, non-volatile media, volatile media and transmission media. Non-volatile media include, for example, optical, magnetic, and solid-state disks, such as a fixed disk. Volatile media include dynamic memory, such as system random-access memory (RAM). Transmission media include coaxial cables, copper wire and fiber optics, among others, including the wires that comprise one embodiment of a bus. Transmission media can also take the form of acoustic or light waves, such as those generated during radio frequency (RF) and infrared (IR) data communications. Common forms of computer-readable media include, for example, a floppy disk, a flexible disk, a hard disk, magnetic tape, any other magnetic medium, a CD-ROM disk, digital video disk (DVD), any other optical medium, any other physical medium with patterns of marks or holes, a RAM, a programmable read-only memory (PROM), an erasable programmable read-only memory (EPROM), an electrically erasable programmable read-only memory (EEPROM), a Flash memory, any other memory chip or data exchange adapter, a carrier wave, or any other medium from which a computer can read.

Various forms of computer-readable media may be involved in carrying one or more sequences of one or more instructions to a CPU for execution. A bus carries the data to system RAM, from which a CPU retrieves and executes the instructions. The instructions received by system RAM can optionally be stored on a fixed disk either before or after execution by a CPU.

Computer program code for carrying out operations for aspects of the present technology may be written in any combination of one or more programming languages, including an object oriented programming language such as JAVA, SMALLTALK, C++ or the like and conventional procedural programming languages, such as the "C" programming language or similar programming languages. The program code may execute entirely on the user's computer, partly on the user's computer, as a stand-alone software package, partly on the user's computer and partly on a remote computer or entirely on the remote computer or server. In the latter scenario, the remote computer may be connected to the user's computer through any type of wired and/or wireless network, including a (wireless) local area network (LAN/WLAN) or a (wireless) wide area network (WAN/WWAN), or the connection may be made to an external computer (for example, through the Internet using an Internet Service Provider, wireless Internet provider, and the like).

The corresponding structures, materials, acts, and equivalents of all means or step plus function elements in the claims below are intended to include any structure, material, or act for performing the function in combination with other claimed elements as specifically claimed. The description of the present technology has been presented for purposes of illustration and description, but is not intended to be exhaustive or limited to the invention in the form disclosed. Many modifications and variations will be apparent to those of ordinary skill in the art without departing from the scope and spirit of the invention. Exemplary embodiments were chosen and described in order to best explain the principles of the present technology and its practical application, and to enable others of ordinary skill in the art to understand the invention for various embodiments with various modifications as are suited to the particular use contemplated.

Aspects of the present technology are described above with reference to flowchart illustrations and/or block diagrams of methods, apparatus (systems) and computer program products according to embodiments of the invention. It will be understood that each block of the flowchart illustrations and/or block diagrams, and combinations of blocks in the flowchart illustrations and/or block diagrams, can be implemented by computer program instructions. These computer program instructions may be provided to a processor of a general purpose computer, special purpose computer, or other programmable data processing apparatus to produce a machine, such that the instructions, which execute via the processor of the computer or other programmable data processing apparatus, create means for implementing the functions/acts specified in the flowchart and/or block diagram block or blocks.

These computer program instructions may also be stored in a computer readable medium that can direct a computer, other programmable data processing apparatus, or other devices to function in a particular manner, such that the instructions stored in the computer readable medium produce an article of manufacture including instructions which implement the function/act specified in the flowchart and/or block diagram block or blocks.

The computer program instructions may also be loaded onto a computer, other programmable data processing apparatus, or other devices to cause a series of operational steps to be performed on the computer, other programmable apparatus or other devices to produce a computer implemented process such that the instructions which execute on the computer or other programmable apparatus provide processes for implementing the functions/acts specified in the flowchart and/or block diagram block or blocks.

The flowchart and block diagrams in the Figures illustrate the architecture, functionality, and operation of possible implementations of systems, methods and computer program products according to various embodiments of the present technology. In this regard, each block in the flowchart or block diagrams may represent a module, segment, or portion of code, which comprises one or more executable instructions for implementing the specified logical function(s). It should also be noted that, in some alternative implementations, the functions noted in the block may occur out of the order noted in the figures. For example, two blocks shown in succession may, in fact, be executed substantially concurrently, or the blocks may sometimes be executed in the reverse order, depending upon the functionality involved. It will also be noted that each block of the block diagrams and/or flowchart illustration, and combinations of blocks in the block diagrams and/or flowchart illustration, can be implemented by special purpose hardware-based systems that perform the specified functions or acts, or combinations of special purpose hardware and computer instructions.

The description of the present technology has been presented for purposes of illustration and description, but is not intended to be exhaustive or limited to the invention in the form disclosed. Many modifications and variations will be apparent to those of ordinary skill in the art without departing from the scope and spirit of the invention. Exemplary embodiments were chosen and described in order to best explain the principles of the present technology and its practical application, and to enable others of ordinary skill in the art to understand the invention for various embodiments with various modifications as are suited to the particular use contemplated.

We claim:

1. A method for non-invasive measurement of biological analytes comprising:

illuminating an analyte using first light, the first light having a first excitation wavelength;

detecting a first spectrum from the analyte illuminated by the first light, the first spectrum including a first Raman signal and fluorescence;

illuminating the analyte using second light, the second light having a second excitation wavelength, the second excitation wavelength being larger than the first excitation wavelength by a first predetermined increment;

detecting a second spectrum from the analyte illuminated by the second light, the second spectrum including a second Raman signal and the fluorescence, the detecting using a Raman spectrometer, the second Raman signal being shifted from the first Raman signal by a second predetermined increment;

illuminating the analyte using third light, the third light having a third excitation wavelength, the third excitation wavelength being larger than the second excitation wavelength by the first predetermined increment;

detecting a third spectrum from the analyte illuminated by the third light, the third spectrum including a third Raman signal and the fluorescence, the third Raman signal being shifted from the second Raman signal by the second predetermined increment;

recovering the first Raman signal using the first spectrum, the second spectrum, and the third spectrum using an inverse transform; and

using the first Raman signal to identify and measure at least one molecule of the analyte using a database of identified Raman signals.

2. The method of claim **1**, wherein the first excitation wavelength, the second excitation wavelength, and the third excitation wavelength are each within a range from ultraviolet light to near infrared light.

3. The method of claim **2**, wherein the first excitation wavelength, the second excitation wavelength, and the third excitation wavelength are each within a range from 650 nm to 950 nm.

4. The method of claim **1**, wherein the first light, the second light, and the third light are provided by a monochromatic light source.

5. The method of claim **4**, wherein the monochromatic light source is a tunable laser.

6. The method of claim **1**, wherein the analyte is at least one of living plant and animal tissue.

7. The method of claim **1**, wherein the analyte is a living human limb.

8. The method of claim **7**, wherein the illuminating the analyte using first light, the detecting the first spectrum, the illuminating the analyte using second light, the detecting the second spectrum, the illuminating the analyte using third light, and the detecting the third spectrum are collectively performed in 25 seconds or less.

9. The method of claim **8**, wherein the at least one molecule is one or more of blood sugar, cholesterol, and a cancer biomarker.

10. The method of claim **1**, wherein the recovering includes iteratively applying expectation maximization techniques.

11. A system for non-invasive measurement of biological analytes comprising:

a monochromatic light source, the monochromatic light source:

illuminating an analyte using first light, the first light having a first excitation wavelength;

illuminating the analyte using second light, the second light having a second excitation wavelength, the second excitation wavelength being larger than the first excitation wavelength by a first predetermined increment; and

illuminating the analyte using third light, the third light having a third excitation wavelength, the third excitation wavelength being larger than the second excitation wavelength by the first predetermined increment;

a Raman spectrometer, the Raman spectrometer:

detecting a first spectrum from the analyte illuminated by the first light, the first spectrum including a first Raman signal and fluorescence;

detecting a second spectrum from the analyte illuminated by the second light, the second spectrum including a second Raman signal and the fluorescence, the detecting using a Raman spectrometer, the second Raman signal being shifted from the first Raman signal by a second predetermined increment; and

detecting a third spectrum from the analyte illuminated by the third light, the third spectrum including a third Raman signal and the fluorescence, the third Raman signal being shifted from the second Raman signal by the second predetermined increment; and

a processor, the processor:

recovering the first Raman signal using the first spectrum, the second spectrum, and the third spectrum using an inverse transform; and

using the first Raman signal to identify and measure at least one molecule of the analyte using a database of identified Raman signals.

12. The system of claim **11**, wherein the first excitation wavelength, the second excitation wavelength, and the third excitation wavelength are each within a range from ultraviolet light to near infrared light.

13. The system of claim **12**, wherein the first excitation wavelength, the second excitation wavelength, and the third excitation wavelength are each within a range from 650 nm to 950 nm.

14. The system of claim **11**, wherein the monochromatic light source is a tunable laser.

15. The system of claim **11**, wherein the analyte is at least one of living plant and animal tissue.

16. The system of claim **11**, wherein the analyte is a living human limb.

17. The system of claim 16, wherein the illuminating the analyte using first light, the detecting the first spectrum, the illuminating the analyte using second light, the detecting the second spectrum, the illuminating the analyte using third light, and the detecting the third spectrum are collectively performed in 25 seconds or less.

18. The system of claim 17, wherein the at least one molecule is one or more of blood sugar, cholesterol, and a cancer biomarker.

19. The system of claim 11, wherein the recovering includes iteratively applying expectation maximization techniques.

20. A system for non-invasive measurement of biological analytes comprising:

means for illuminating an analyte using first light, the first light having a first excitation wavelength;

means for detecting a first spectrum from the analyte illuminated by the first light, the first spectrum including a first Raman signal and fluorescence;

means for illuminating the analyte using second light, the second light having a second excitation wavelength, the second excitation wavelength being larger than the first excitation wavelength by a first predetermined increment;

means for detecting a second spectrum from the analyte illuminated by the second light, the second spectrum including a second Raman signal and the fluorescence, the detecting using a Raman spectrometer, the second Raman signal being shifted from the first Raman signal by a second predetermined increment;

means for illuminating the analyte using third light, the third light having a third excitation wavelength, the third excitation wavelength being larger than the second excitation wavelength by the first predetermined increment;

means for detecting a third spectrum from the analyte illuminated by the third light, the third spectrum including a third Raman signal and the fluorescence, the third Raman signal being shifted from the second Raman signal by the second predetermined increment;

means for recovering the first Raman signal using the first spectrum, the second spectrum, and the third spectrum using an inverse transform; and

means for using the first Raman signal to identify and measure at least one molecule of the analyte using a database of identified Raman signals.

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